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Synthesis and *In Vitro* Autoradiographic Evaluation of a Novel High-affinity Radioiodinated Ligand for Imaging Brain Cannabinoid Subtype-1 Receptors

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Abstract

There is strong interest to study the involvement of brain cannabinoid subtype-1 ($CB_{1)}$ receptors in neuropsychiatric disorders with single photon emission computed tomography (SPECT) and a suitable radioligand. Here we report the synthesis of a novel high-affinity radioiodinated CB_1 receptor ligand ([^{125}I]**8**, [^{125}I]1-(2-iodophenyl)-4-cyano-5-(4-methoxyphenyl)-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxylate, [^{125}I]SD7015). By autoradiography in vitro, [^{125}I]**8** showed selective binding to CB_1 receptors on human brain postmortem cryosections and now merits labeling with iodine-123 for further evaluation as a SPECT radioligand in non-human primate.

Keywords

SPECT; PET; CB_1 receptors; radioiodination; radioligand

Cannabis sativa (marijuana) is one of the oldest known plant derived-therapeutics, owing mainly to its anti-nociceptive properties.[1] Other beneficial effects of cannabis intake may include anti-emesis and appetite stimulation. Conversely, the accompanying psychological "high" and memory impairment have limited its therapeutic value. The most abundant and psychoactive cannabinoid, Δ^9-tetrahydrocannabinol (Δ^9-THC, **1**), binds with high affinity to two known body receptor systems. These receptors have been named cannabinoid subtype-1 (CB_1) and cannabinoid subtype-2 (CB_2).[2–4] CB_1 receptors have high-densities in brain and are implicated in a number of neuropsychiatric disorders[5] such as, schizophrenia[6,7] and depression[8,9]. CB_2 receptors are located mainly in the periphery and are of less interest to neuropsychiatric research.[10,11]

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Ability to image and measure brain CB_1 receptors non-invasively with radiation computed tomography would assist neuropsychiatric research and drug development. The development of radioligands suitable for imaging brain CB_1 receptors is therefore of strong importance. Recently, there has been a great deal of progress in the development of useable radioligands for positron emission tomography (PET). Some of the more notable PET radioligands (Figure 1) are [^{11}C]MePPEP ([^{11}C]**2**)[12,13], [^{18}F]PipISB ([^{18}F]**3**)[14,15], [^{18}F]MK-9470 ([^{18}F]**4**)[16,17], (−)-[^{11}C]SD5014 ((−)-[^{11}C]**5**)[18], [^{11}C]OMAR ([^{11}C]**6**)[19,20], and [^{11}C]JHU75575 ([^{11}C]**7**)[20,21]. In comparison, the development of radioligands for more widespread single-photon computed tomography (SPECT) is much less advanced. Due to the longer half-life of radioiodine for SPECT imaging (e.g., ^{123}I, $t_{1/2}$ = 13.2 h), an on-site cyclotron is not required for radiolabeling, as with PET, which allows for more widespread use in pharmaceutical, academic and hospital settings. Here we report the synthesis, receptor screening, radioiodination and in vitro autoradiographic evaluation of a novel radioiodinated CB_1 receptor radioligand ([^{125}I]4-cyano-1-(2-iodophenyl)-5-(4-methoxyphenyl)-*N*-(piperidin-1-yl)-1*H*-pyrazole-3-carboxamide, [^{125}I]**8**, [^{125}I]SD7015). Our results strongly suggest that [^{123}I]**8** would merit evaluation as a SPECT radioligand in vivo.

Compound **8** was synthesized according to the procedure depicted in Scheme 1. Conversion of 2-iodoaniline into a diazonium salt, followed by treatment with ethyl 2-chloroacetoacetate in ethanol-water solution under basic conditions gave chloro[(2-iodophenyl)hydrazono]ethyl acetate (**9**). Heating of a solution of **9**, 4-methoxybenzoylacetonitrile and *N*,*N*-diisopropylethylamine in *tert*-butanol to reflux gave **10** in low but adequate yield. Hydrolysis of **10** with LiOH in *aq*-tetrahydrofuran gave the corresponding carboxylic acid, which was then converted into the acyl chloride with oxalyl chloride and a catalytic amount of *N*,*N*-dimethylformamide. The synthesis of **8** was completed by coupling the acyl chloride with 1-aminopiperidine under basic conditions.

The trimethylstannylated precursor, **11**, required for radiolabeling, was synthesized by refluxing a solution of the known bromo compound **7**[20] and hexamethylditin in the presence of palladium catalyst (Scheme 2)

A radioligand for imaging brain CB_1 receptors would ideally possess adequately high affinity and moderate lipophilicity to promote rapid development of a high ratio of receptor-specific binding to non-specific binding in brain in vivo, that might then allow accurate computation of output measures, such as binding potential.[22,23] CB_1 receptors are one of the most abundant G protein-coupled receptors in brain, reaching a concentration (B_{max}) of 1752 fmol/mg protein (175 nM) in rat cerebellum.[24] Generally, a SPECT radioligand should show a substantial B_{max}/K_d value (> 5) to be successful. Hence, an effective CB_1 receptor radioligand might require only a moderately high affinity (K_i or K_d < 35 nM). Ligand **8** was found to have about ten-fold higher affinity (3.4 nM; Table 1) than perhaps needed. Calculated ligand lipophilicity ($cLogD_{7.4}$) can be an important predictor of blood–brain barrier penetration and brain non-specific binding. Moderate lipophilicity ($cLogD_{7.4}$ in the range 2.0 – 3.5) is usually preferred for adequate brain entry without incurring excessive non-specific binding in brain.[23] However, when target binding sites exist in high concentration, higher lipophilicity may be tolerated. For example, [^{11}C]MePPEP is a successful PET radioligand for brain CB_1 receptors despite having a high $cLogD_{7.4}$ value of 5.42.[12,13] The $cLogD_{7.4}$ value of **8** is 4.14 and appears more favorable for a radioligand than that of [^{11}C]MePPEP. Futhermore, the physiochemical and pharmacological properties of **8** compare well with other successful PET radioligands.[20,21] Therefore, ligand **8** presents acceptable CB_1 receptor affinity and lipophilicity for development as a SPECT radioligand (Table 1).

In addition to acceptable binding affinity and lipophilicity a candidate SPECT radioligand should be selective for binding to the target protein. Ligand **8**, at 10 μM concentration, showed

< 50% inhibition (n = 4) of radioligand binding to the sites: 5-HT_{1B-E}, 5-HT_{2A-C}, 5-HT_3, 5-HT_{5A}, 5-HT_6, 5-HT_7, $\alpha_{1A,B}$, $\alpha_{2A,B}$,β_{1-3}, D_{1-4}, DOR, H_{1-4}, M_{1-5}, NET, SERT,$\sigma_{1,2}$, $V_{1A,1B,2}$. K_i values (n = 3) of 93.5 ± 20.4 nM (5-HT_{1A}), > 10,000 nM (α_{2C}), 1,477 ± 148 nM (KOR), 1,496 ± 216 nM (MOR) and 3,166 ± 586 nM (TSPO) were found. Details of the employed binding assays may be found at the NIMH PDSP web site: http://pdsp.med.unc.edu. Hence, **8** was found to have excellent CB_1 receptor selectivity for development as a SPECT radioligand.

[^{125}I]**8** was prepared by [^{125}I]iodo-destannylation of the corresponding trimethylstannyl precursor (**11**) with [^{125}I]NaI, *aq*-HCl and chloramine-T (oxidizer) in methanol (Scheme 2). The crude product was purified with high-performance liquid chromatography (HPLC) as described in Supplementary Information. The decay-corrected radiochemical yield of [^{125}I]**8** ranged from 48 to 59%. The specific radioactivity of [^{125}I]**8** was 81.4 GBq/μmol and the radiochemical purity > 98%. [^{125}I]**8** was thus obtained in adequate yield and purity for further evaluation with sensitive post mortem autoradiography in vitro. Furthermore, these conditions would be applicable to labeling **8** with iodine-123 for evaluation in SPECT imaging.

CB_1 receptors are spread heterogeneously in brain, with high-densities appearing in substantia nigra, globus pallidus, amygdala, cortical regions and striatum.[25,26] Brain regions with low CB_1 receptor densities are thalamus, pons and white matter. In post mortem autoradiography in vitro, [^{125}I]**8** bound substantially to human brain regions with high CB_1 receptor densities (Figure 2; Panels A, C, E & G) with highest binding in globus pallidus and substantia nigra (Figure 2; Panels A & E). Additionally [^{125}I]**8** showed much lower binding in brain regions with low CB_1 receptor density, including thalamus (Figure 2; Panels C & E). Under conditions in which the CB_1 receptors were blocked with the selective CB_1 ligand, rimonabant (10 μM), the binding of [^{125}I]**8** in CB_1 rich regions was greatly reduced and the distribution of radioligand became more homogeneous (Figure 2, Panels B, D, F & H). Therefore, the regional selectivity of [^{125}I]**8** indicated that ^{123}I-labeled **8** would be promising for imaging brain CB_1 receptors with SPECT.

Ligand **8** demonstrated high affinity and good selectivity for CB_1 receptors. [^{125}I]**8** was obtained in acceptable radiochemical yield, specific radioactivity and purity for evaluation *in vitro*. Autoradiographs of human brain obtained with [^{125}I]**8** showed radioactivity distribution according to known regional CB_1 receptor densities. Future research evaluating [^{123}I]**8** with SPECT imaging is therefore warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Lambert DM. J Pharm Belg 2001;56:111. [PubMed: 11759149]
2. Devane WA, Dysarz FA, Johnson MR, Melvin LS, Howlett AC. Mol Pharmacology 1988;34:605.
3. Munro S, Thomas KL, Abushaar M. Nature 1993;365:61. [PubMed: 7689702]

4. Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, Felder CC, Herkenham M, Mackie K, Martin BR, Mechoulam R, Pertwee RG. Pharmacol Rev 2002;54:161. [PubMed: 12037135]
5. Van Laere K. Eur J Nucl Med Mol Imaging 2007;34:1719. [PubMed: 17643242]
6. Dean B, Sundram S, Bradbury R, Scarr E, Copolov D. Neurosci 2001;103:9.
7. Eggan SM, Hashimoto T, Lewis DA. Arch Gen Psychiatry 2008;65:772. [PubMed: 18606950]
8. Serra G, Fratta W. Clin Pract Epidemol Ment Health 2007;3:25. [PubMed: 18021439]
9. Zavitsanou K, Garrick T, Huang XF. Prog Neuropschyopharmacol Biol Psychiatry 2004;28:355.
10. Lynn AB, Herkenham M. J Pharmacol Exp Ther 1994;268:1612. [PubMed: 8138973]
11. Gong JP, Onaivi ES, Ishiguro H, Liu QR, Tagliaferro PA, Brusco A, Uhl GR. Brain Res 2006;1071:10. [PubMed: 16472786]
12. Yasuno F, Brown AK, Zoghbi SS, Krushinski JH, Chernet E, Tauscher J, Schaus JM, Phebus LA, Chesterfield AK, Felder CC, Gladding RL, Hong J, Halldin C, Pike VW, Innis RB. Neuropsychopharmacology 2008;33:259. [PubMed: 17392732]
13. Donohue SR, Krushinski JH, Pike VW, Chernet E, Chesterfield AK, Felder CC, Halldin C, Schaus JM. J Med Chem 2008;51:5833. [PubMed: 18800770]
14. Donohue SR, Halldin C, Schou M, Hong J, Phebus LA, Chernet E, Hitchcock SA, Gardinier KM, Ruley KM, Krushinski JH, Schaus JM, Pike VW. J Labelled Compd Radiopharm 2008;51:149.
15. Finnema SJ, Donohue SR, Zoghbi SS, Brown AK, Gulyás B, Innis RB, Halldin C, Pike VW. Synapse 2009;63:22. [PubMed: 18925657]
16. Burns HD, Van Laere K, Sanabria-Bohorquez S, Hamill TG, Bormans G, Eng WS, Gibson R, Ryan C, Connolly B, Patel S, Krause S, Vanko A, Van Hecken A, Dupont P, De Lepeleire I, Rothenberg P, Stoch SA, Cote J, Hagmann WK, Jewell JP, Lin LS, Liu P, Goulet MT, Gottesdiener K, Wagner JA, de Hoon J, Mortelmans L, Fong TM, Hargreaves RJ. Proc Natl Acad Sci USA 2007;104:9800. [PubMed: 17535893]
17. Liu P, Lin LS, Hamill TG, Jewell JP, Lanza TJ, Gibson RE, Krause SM, Ryan C, Eng WS, Sanabria S, Tong XC, Wang JY, Levorse DA, Owens KA, Fong TM, Shen CP, Lao JL, Kumar S, Yin WJ, Payack JF, Springfield SA, Hargreaves R, Burns HD, Goulet MT, Hagmann WK. J Med Chem 2007;50:3427. [PubMed: 17608398]
18. Donohue SR, Pike VW, Finnema SJ, Truong P, Andersson J, Gulyás B, Halldin C. J Med Chem 2008;51:5608. [PubMed: 18754613]
19. Horti AG, Fan H, Kuwabara H, Hilton J, Ravert HT, Holt DP, Alexander M, Kumar A, Rahmim A, Scheffel U, Wong DF, Dannals RF. J Nucl Med 2006;47:1689. [PubMed: 17015906]
20. Fan H, Ravert HT, Holt DP, Dannals RF, Horti AG. J Labelled Compd Radiopharm 2006;49:1021.
21. Donohue SR, Zoghbi S, Yasuno F, Terry G, Gourley J, Innis RB, Halldin C, Pike VW. Curr Radiopharmaceuticals 2008;1:93.
22. Laruelle M, Slifstein M, Huang Y. Mol Imaging Biol 2003;5:363. [PubMed: 14667491]
23. Waterhouse RN. Mol Imaging Biol 2003;5:376. [PubMed: 14667492]
24. Hirst RA, Almond SL, Lambert DG. Neurosci Lett 1996;220:101. [PubMed: 8981483]
25. Herkenham M, Lynn AB, Johnson MR, Melvin LS, De Costa BR, Rice KC. J Neurosci 1991;11:563. [PubMed: 1992016]
26. Glass M, Dragunow M, Faull RL. Neuroscience 1997;77:299. [PubMed: 9472392]

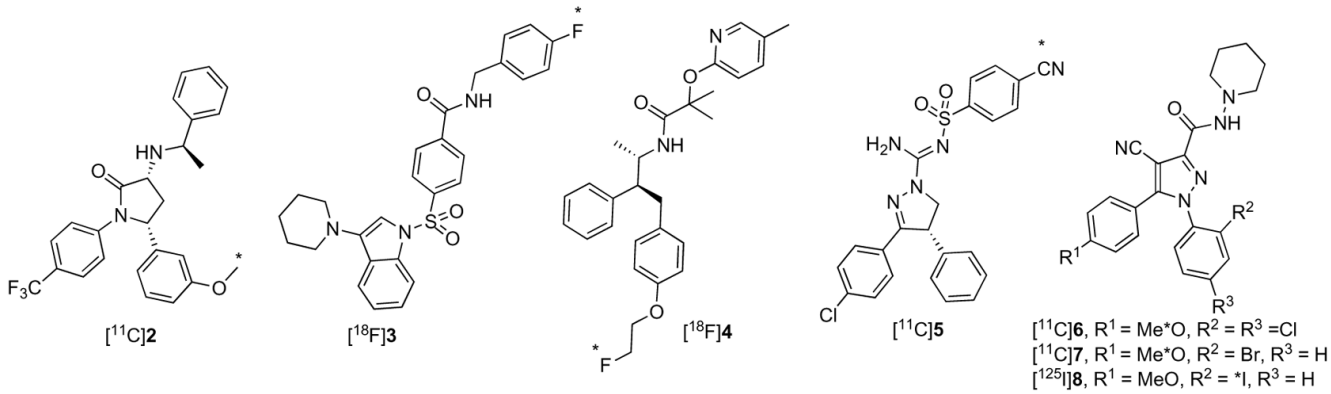


Figure 1.
Structures of [^{11}C]**2**, [^{18}F]**3**, [^{18}F]**4**, [^{11}C]**5– 7**, and [^{125}I]**8**. Asterisks denote positions of radiolabels.

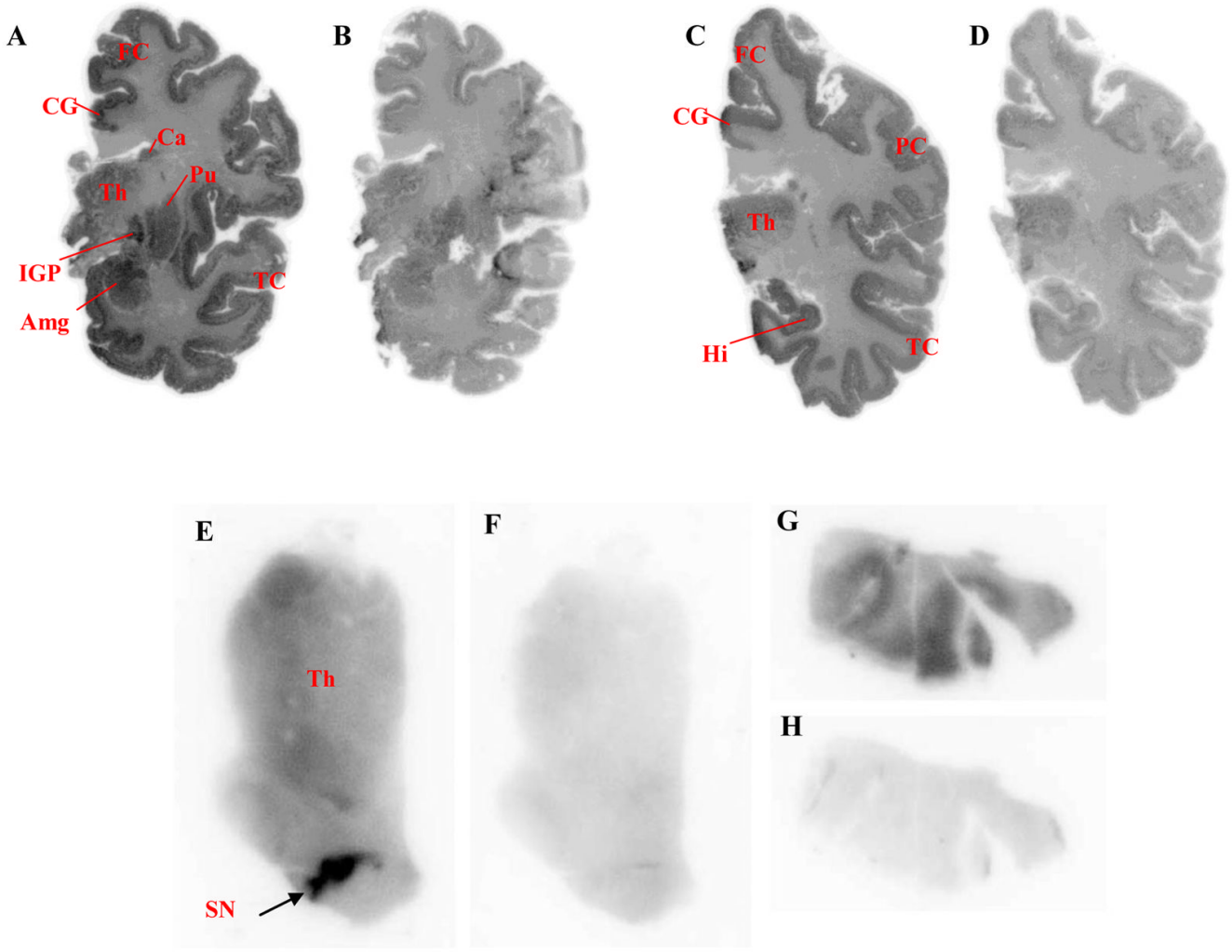
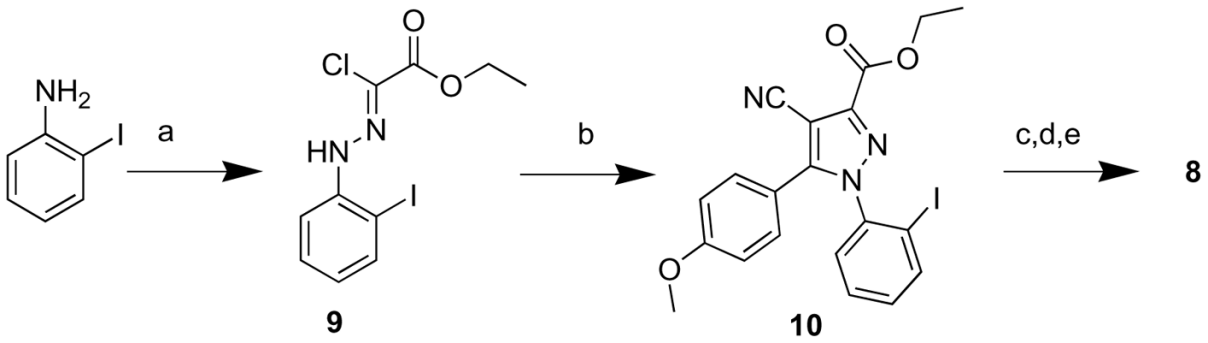


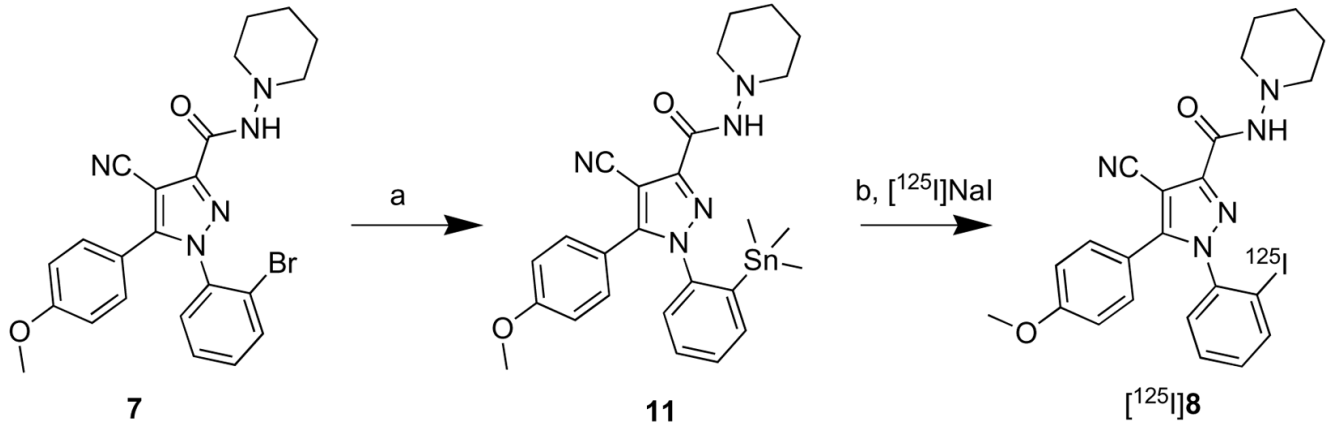
Figure 2.

Autoradiographs from whole-hemisphere cryosections (Panels A– D; 100 m thickness) and cryosections (20 μm thickness) covering thalamus and brainstem (Panels E & F) and temporal cortex (Panels G & H) incubated with [^{125}I]**8** under baseline (Panels A, C, E & G) and blocked (rimonabant, 10 μM; Panels B, D, F & H) conditions. Abbreviations: Amg, amygdala; Ca, caudate nucleus; CG, cingulate gyrus; FC, frontal cortex; Hi, hippocampus; IGP, globus pallidus, internal segment; PC, parietal cortex; Pu, putamen; SN, substantia nigra; TC, temporal cortex; Th, thalamus.



Scheme 1.

Synthesis of **8**. Reagent, conditions and yields: a) concentrated HCl, $NaNO_2$, ethyl 2-chloroacetoacetate, NaOAc, EtOH– H_2O, 16 h, 72%; b) 4-methoxybenzoylacetonitrile, DIPEA, *tert*-BuOH, reflux, 16 h, 6%; c) *aq*-LiOH, THF, 65 °C, 4 h; d) $DMF_{(cat)}$, $(COCl)_2$, DCM; e) 1-aminopiperidine, DIPEA, DCM, 2 h, 73%.

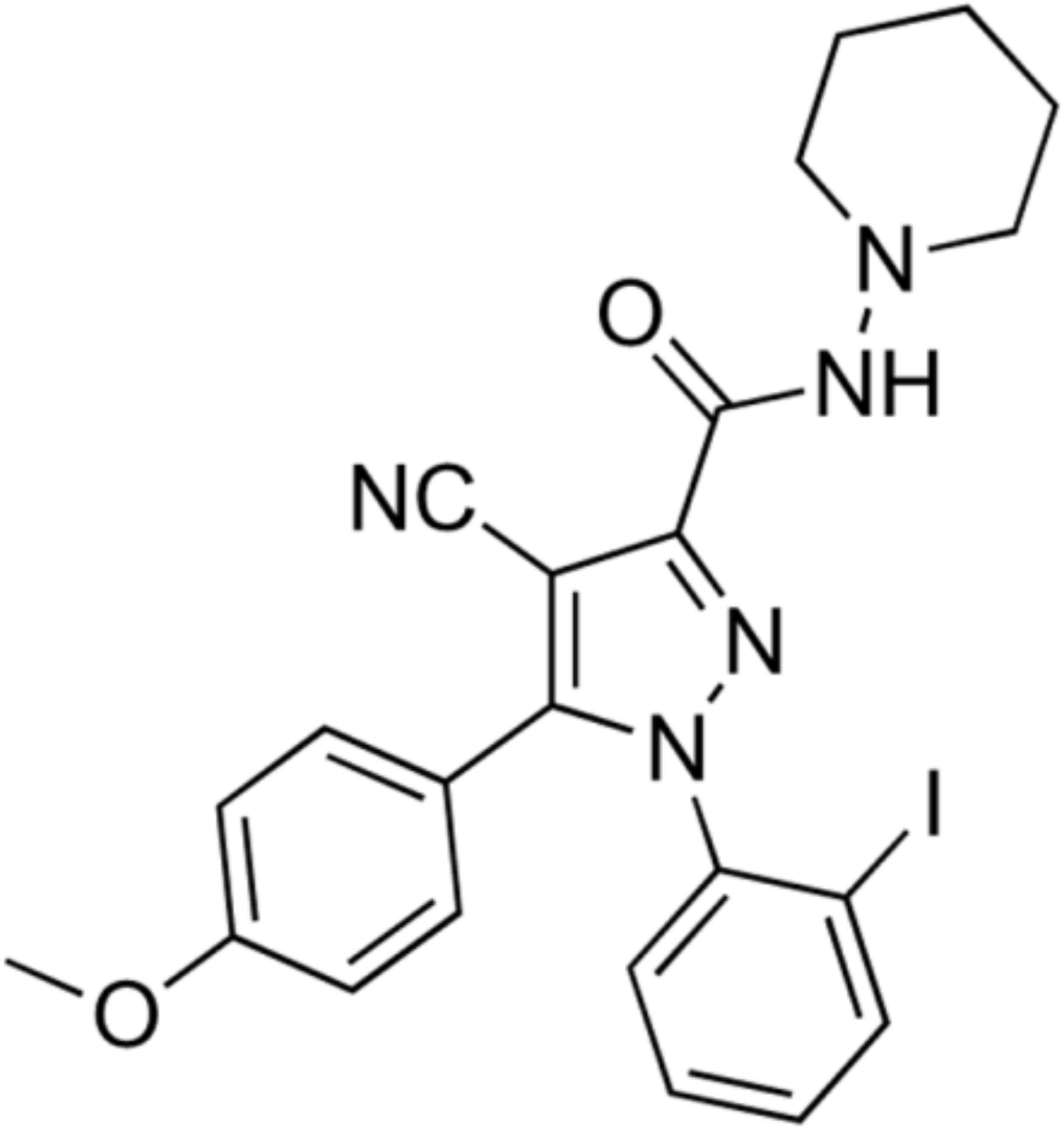


Scheme 2.

Radiosynthesis of [^{125}I]**8**. Reagents, conditions and yields: a) hexamethylditin, $Pd(PPh_3)_4$, toluene, 20 h, 32%; b) [^{125}I]NaI, chloramine- T, *aq*-HCl, 5 min, 48– 59%.

Table 1

K_i values for CB_1 and CB_2 receptors, selectivities for CB_1 versus CB_2 receptors and calculated lipophilicities.



Ligand	CB_1K_i (nM)[a]	CB_2K_i (nM)[a]	CB_1 versus CB_2 selectivity[a]	$cLogD_{7.4}$[b]
rimonabant	1.38 ± 0.17	927 ± 66	672 ± 94	6.01
8	3.40 ± 0.43	548 ± 23	161 ± 47	4.14

[a] Values represent mean ± SD of three determinations.

[b] *cLogD7.4* values were calculated using Advanced Chemistry Development (ACD) 9.2.